Inbreeding Depression in Insular and Central Populations of *Peromyscus* Mice

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We tested the hypothesis that small, isolated populations would show less depression in fitness when inbred than would large, central populations. Laboratory stocks of *Peromyscus leucopus* and *P. polionotus* were established from insular, peninsular, and central populations. The isolated populations had one-third to one-half the genic diversity of central populations. Responses to inbreeding were highly varied: some populations had smaller litters, others experienced higher mortality, some showed slower growth rates, and one displayed no measurable effects when inbred. These results suggest that inbreeding depression is controlled by a small number of genes and that the size of the genetic load depends on which alleles are present in the founders of a population. The severity of fitness depression in inbred litters did not correlate with initial genic diversity of the stocks nor, therefore, with the size of the wild populations. Fitness measures appeared linearly related to the inbreeding coefficient of the liters, with no diminution of deleterious effects through subsequent generations of inbreeding. Thus overdominance of fitness traits probably contributed as much to the genetic load as did deleterious recessive alleles. The inbreeding level of the dam negatively affected the size, growth, and survival of litters only in genetically diverse populations, indicating that the load of recessive alleles negatively impacting maternal care may have been reduced by selection in the more peripheral populations during past bottlenecks.

Darwin[11] presented a large body of data documenting inbreeding depression in domesticated stocks. Wright[57] and Falconer[13] summarized subsequent experimental work, showing that inbreeding in normally outbreeding species generally results in a decline in characters associated with fitness. Yet studies of inbreeding in natural populations of vertebrates, and even studies of recently established captive stocks, are limited to a relatively few species and may have yielded conflicting results (e.g., great tits;[18,54] house mice;[9,26] deermice;[12,19,22,50] baboons[8,37]). Ralls and colleagues compiled data on juvenile mortality in ungulates,[41] small mammals,[38] and primates[39] in zoos. Although the data obtainable from zoo records were uncontrolled, they were sufficient to demonstrate that inbred offspring suffered greater mortality than did noninbred offspring in many species. Ralls et al.[40] examined the association between inbreeding and juvenile mortality in captive populations of mammals, finding a positive relationship in 36 of 40 taxa, but with considerable variation between taxa in the severity of inbreeding depression observed.

Inbreeding leads to greater homozygosity of individuals, and the more frequent phenotypic expression of deleterious, recessive alleles in inbred organisms is though to be a major cause of inbreeding depression.[13,57] The number of "lethal equivalents" per diploid individual has been estimated at about two to five for human populations.[29,32] Ralls et al.[40] reported a median of 3.1 lethal equivalents for 40 mammalian populations.

Inbreeding depression may also result from the presence of loci that show overdominance for fitness effects. Lerner[24] presented the case for general heterosis resulting from greater developmental stability in more heterozygous individuals, and subsequent workers have shown developmental homeostasis in a variety of organisms.[31]

Inbreeding depression is not an inevitable correlate of close mating. Shields[47] proposed that many natural populations are philopatric and thus experience, and are adapted to, local inbreeding. Some animals and about one-third of plants regularly inbreed without marked problems.[21,45] Rao and Inbaraj[42] found no effect

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of inbreeding on fetal growth and development in a population of humans with a long-term practice of inbreeding. Successful inbred stocks of several species have been developed, although they may have suffered initially high inbreeding depression.[49,51] The mitigation of inbreeding effects in species that commonly inbreed is most easily explained by the removal by natural selection of deleterious recessives, but may also result in part from selection for modifiers that reduce the genetic load of heterotic loci. In either case, selection would be expected to lead to a genome with a small genetic load (relatively adapted to homozygosity) after generations of forced inbreeding, but the direct selective removal of deleterious recessives would be more efficient than indirect selection to counter overdominance. Healthy inbred stocks of laboratory mice attest to the ability of selection to produce vertebrate genomes with little genetic load, but the lower fitness of those stocks relative to outbred crosses as well as the difficulty researchers have experienced in producing fully inbred strains of many other species demonstrate the incomplete efficiency of selection to remove the genetic load. Templeton and Read[52] reported a decrease in the lethal equivalents (from 6.18 to 1.22) resulting from inbreeding of Speke's gazelles, an endangered species of North African antelope. This marked reduction of the genetic load is much greater than could be accounted for by selection against recessives (the maximum inbreeding coefficient attained was .31) but, as Templeton and Read point out, small sample sizes precluded rigorous statistical analysis.

The ability of selection to adapt a population to individual homozygosity will affect the fate of populations that go through bottlenecks. Although little electrophoretically detectable variation has been observed in several populations that are known to have experienced population crashes (e.g., northern elephant seals;[6] black-footed ferrets[36]), it is not known whether such populations became less susceptible to inbreeding depression. To test whether natural populations that have experienced variance-depleting bottlenecks tolerate inbreeding with fewer deleterious effects, we examined the response of insular, peninsular, and mainland populations of two species of *Peromyscus* mice (family Cricetidae). We predicted that more isolated and narrowly endemic populations would show less electrophoretically detectable variation in protein-coding loci and would experience less depression of fitness traits when forced to inbreed in the laboratory.

Table 1. Taxa, collecting sites, numbers of mice collected, numbers of wild-caught mice that contributed to the lab stocks, and population abbreviations for the founders used to establish laboratory stocks

Taxon	No. captured/bred	Collecting locality	Abbreviation
Island populations			
P. polionotus phasma	9/6	Anastasia State Park, Florida	I-ANA
P. p. leucocephalus	11/7	Santa Rosa Island, Florida	I-SRI
Peripheral populations			
P. p. rhoadsi	11/10	Lake Placid, Florida	P-LP
P. p. niveiventris	15/15	Canaveral National Seashore, Florida	P-CNS
Central populations			
P. p. subgriseus	15/10	Ocala National Forest, Florida	C-ONF
P. p. subgriseus	12/8	Levy County, Florida	C-LVY
P. leucopus tornillo	10/9	Brewster, Presidio, Jeff Davis counties, Texas	C-TX
P. l. noveboracensis	5/5	Ithaca, New York	C-NY

Materials and Methods

Laboratory populations were established from six populations representing five subspecies of *P. polionotus* (coastal populations commonly called "beach mice," inland populations called "oldfield mice") collected in Florida (January 1984), and from two populations of separate subspecies of *P. leucopus* (white-footed mice) collected in New York (July 1983) and Texas (May 1983). Taxa, collecting sites, and numbers of mice collected are given in Table 1.

The populations sampled represent a gradient of range contraction and isolation. *Peromyscus polionotus leucocephalus* were collected from the frontal dunes and from nearby grassy fields at the western end of Santa Rosa Island, Escambia County, Florida. (This population will be designated I-SRI; the prefix "I" denoting an insular population.) Although always endemic to Santa Rosa Island, habitat destruction and predation by introduced cats now restrict this subspecies to the protected sections of the Gulf Islands National Seashore on the island. Hurricanes occasionally decimate the Gulf Coast populations of beach mice occupying the frontal dunes.[30] Although *P. p. leucocephalus* is not now threatened with extinction, three of five Gulf Coast subspecies are listed as endangered,[53] including those inhabiting keys immediately to the east and immediately to the west of Santa Rosa Island.

P. p. phasma were trapped in Conch Island adjacent to Anastasia State Park, St. Johns County, Florida, in a sand dune habitat (I-ANA population). This subspecies has been largely or totally eliminated from the adjoining mainland owing to habitat destruction, predation by domestic cats, and competition from house mice. The subspecies is now limited to less than 25 acres of remaining habitat and is listed as endangered. *P. p. niveiventris* were taken from a similar habitat in the Canaveral National Seashore (P-CNS; the prefix denoting a peripheral population), Brevard County and Volusia County. Although still common in the frontal dunes of the national seashore, Canaveral Air Force Station, and Kennedy Space Center, this subspecies which once extended from Miami to Daytona is probably now restricted to the protected areas on Cape Canaveral and is listed as threatened. A subspecies (*P. p. decoloratus*) that once inhabited the dunes between *phasma* and *niveiventris* is now extinct.[23]

P. p. rhoadsi were captured from burrows along roadsides in Lake Placid, Highlands County, Florida, at the southern extreme of the species distribution (P-LP population). This subspecies of oldfield mouse occurs in the sandy loam banks and fields along the sand ridge of midcentral Florida. *P. p. subgriseus* were collected from sandy loam pine forest habitat in two localities in north-central Florida: state Highway 42, Altoona, Lake County, along the southern edge of the Ocala National Forest (C-ONF population; prefix denoting a central population) and along U.S. Route 41, Levy County, (C-LVY population). The oldfield mouse is widespread in the extensive open forests and old fields of both areas.

P. leucopus noveboracensis were trapped in Ithaca, Thompkins County, New York (C-NY population). The white-footed mouse is abundant throughout the eastern deciduous forests. *P. l. tornillo* were trapped along a two-mile stretch of railroad right-of-way in Altuda, Brewster County, in the Davis Mountains, Jeff Davis County, and along roadsides in Presidio County, all in southwestern Texas (C-TX population). In

Table 2. Enzyme systems studied, E.C. numbers, and primary buffer systems used for assessment of genic variation in founder stocks

Enzyme	E.C. no.	Buffer*
Acid phosphatase	3.1.3.2	C
Adenosine deaminase	3.5.4.4	C
Adenylate kinase	2.7.4.3	C (2)
Alcohol dehydrogenase	1.1.1.1	4
Aldolase	4.1.2.13	C
Aspartate aminotransferase	2.6.1.1	M (2)
Creatinine kinase	2.7.3.2	R
b-Galactosidase	3.2.1.23	4
b-Glucosidase	3.2.1.21	4
Glutathione reductase	1.6.4.2	4
a-Glycerophosphate dehydrogenase	1.1.1.8	4
Isocitrate dehydrogenase	1.1.1.42	C (2)
Lactate dehydrogenase	1.1.1.27	4 (2)
Malate dehydrogenase	1.1.1.37	4 (2)
Mannosephosphate isomerase	5.3.1.8	M
Methylumbelliferyl phosphatase	3.1.3.2	4
Peptidase—leucyl-alanine	3.4.11-13	R
Peptidase—leucyl-leucyl-leucine	3.4.11-13	R (2)
Peptidase—phenylalanyl-proline	3.4.11-13	R
Phosphoglucomutase	2.7.5.1	R
6-Phosphogluconate dehydrogenase	1.1.1.44	C
Phosphoglucose isomerase	5.3.1.9	4
Sorbitol dehydrogenase	1.1.1.14	4
Superoxide dismutase	1.15.1.1	C

*C = CT buffer of May et al.;[26] M = Markert and Faulhaber;[27] R = Ridgway et al.;[43] 4 = buffer 4 of Selander et al.[46] Number of systems scored (when more than one) on each buffer is given in parentheses.

this area, the white-footed mouse occupies middle- to high-elevation desert scrub habitat. The C-NY and C-TX populations were confirmed to be *P. leucopus*, rather than the sympatric *P. maniculatus*, by examination of external and skull morphology,[20] electrophoretic analysis of salivary amylase,[2] and breeding tests with animals obtained from the *Peromyscus* Stock Center in Columbia, South Carolina. Details of capture localities and methods and of laboratory maintenance procedures are available in Brewer.[7]

All founders of each lab population were assumed to be unrelated regardless of capture site within the trap grid. Initially, mice were paired at random within local populations. Subsequently, each generation some mice were paired with known relatives (sibs, parents, half-sibs, or more complex, multiple relationships), and others were paired with mice that shared no common ancestry within the captive colony. Pairs to be mated were chosen from matrices of genetic relationships, without regard to phenotypes. After several generations of production of inbred and outbred mice, pairings were made between outbred, unrelated parents (noninbred controls), between outbred, related parents (to produce inbred offspring from outbred parents), between inbred, unrelated parents (outbred offspring from inbred parents), and between inbred, related parents (inbred offspring from inbred parents). Additionally, some inbred mice were paired with outbred mice, producing litters of varying degrees of inbreeding. In no case were litters produced from outbred parents that in turn had inbred ancestors within the lab colony (i.e., outbred offspring from inbred parents were never used for breeding). Thus, the inbreeding coefficients of mice used as parents always reflected a steady accumulation of inbreeding in the lab ancestry and therefore also reflected the opportunity for past selection to have removed alleles that contribute to inbreeding depression.

Pairs were left together until parturition or approximately two months if no litter was produced. Sires were removed either just after parturition or when the litter was weighed and weaned at 20 days of age. Dams were left to deliver and rear a second litter (if they had bred during the postpartum estrus). Mice were paired with multiple mates to produce both inbred and outbred litters during their lifetimes. Animals no longer needed for breeding were euthanatized with ether, and tissues (leg muscle, heart muscle, liver, and kidney) were removed and stored at −70°C for electrophoretic analysis.

The initial level of genetic variation in each population was assessed by starch gel electrophoresis of protein variation in tissue samples taken from postmortem, wild-caught mice. All wild-caught founder animals except those that were cannibalized or discovered in an autolyzed condition were analyzed (74 of 88 wild-caught mice). Electrophoresis was carried out on mixed tissue homogenates (liver, heart, muscle, kidney, and blood). Thirty enzyme systems were resolved across all eight populations of mice (Table 2). Electrophoresis methods were similar to those of Selander et al.[46] and are detailed in Brewer.[7] For each population, allozyme variation was summarized by percentage of polymorphic loci, number of allozymes per locus, heterozygosity expected under Hardy-Weinberg equilibrium (corrected for small sample size[34]), and observed (individual) heterozygosity.

Regression Analysis of Inbreeding Effects

Each litter was treated as one independent data point. Litters of wild-caught females were excluded from analysis because the histories of those females were not controlled and ages of wild-caught dams were unknown. Pairings were not rigorously controlled for number of days prior to separation of mates (pairs were left together for six to 12 weeks before separation if no litters had been produced), and no attempt was made to analyze the proportion of inbred vs. outbred pairs that produced litters (fertility). Demographic data collection on each litter included: initial number of offspring, fraction of offspring surviving until weaning at 20 days of age, number of offspring weaned, sex ratio at weaning, mean individual offspring mass at weaning, and total litter mass at weaning.

Inbreeding coefficients of all mice were calculated by the additive matrix method.[3] The response of each population to inbreeding was assessed by multiple regression analysis of each demographic variable against the inbreeding coefficient of the litter, inbreeding coefficient of the dam, inbreeding coefficient of the sire, age of dam at conception, and parity (primiparous vs. multiparous dams). The multivariate generalized linear hypothesis module of the SYSTAT (Systat, Inc., Evanston, Illinois) statistical analysis package was used to estimate coefficients in the multivariate models.

The first litter produced by a female averaged fewer young, higher preweaning mortality, and smaller offspring at weaning than did subsequent litters in all populations. There was no statistically detectable difference in any of the variables measured among second, third, fourth, and later litters. Therefore, the two-level parity effect of first vs. later litters was included in the multivariate regression models. For several of the demographic variables (offspring mass, litter mass, initial litter size), the variance is expected to be correlated with the mean. Therefore, natural log transforms of the demographic variables were applied prior to regression analysis. For those variables that can take on a value of 0 (number weaned, litter viability, litter weight), the logarithm was taken of one plus the raw score. Neither the log transformations nor any other transformations tried (square root, arcsin square root) significantly improved the fit of the data to the regression models, nor did any of the transformations alter the trends (or lack thereof) reported below.

Litter size is a discrete variable that is leptokurtic (most litters consist of three to five offspring). Several other demographic variables (survival to weaning, litter mass at weaning) are strongly bimodal, because entire litters usually live or die. Therefore, it is inappropriate to assess with

Table 3. Measures of genetic variability at 30 allozyme loci in founder stocks

Population	H_e	H_o	P	n_a
I-ANA	.040 (.020)	.042 (.027)	.13 (.06)	1.13 (.06)
I-SRI	.053 (.029)	.029 (.018)	.10 (.06)	1.10 (.06)
P-LP	.087 (.032)	.079 (.035)	.27 (.08)	1.30 (.10)
P-CNS	.088 (.035)	.097 (.038)	.20 (.07)	1.27 (.11)
C-ONF	.075 (.027)	.060 (.022)	.30 (.08)	1.37 (.11)
C-LVY	.126 (.038)	.106 (.035)	.33 (.09)	1.47 (.14)
C-TX	.106 (.034)	.071 (.029)	.33 (.09)	1.37 (.10)
C-NY	.112 (.039)	.117 (.044)	.23 (.08)	1.30 (.11)

H_e, mean expected heterozygosity; H_o, mean observed heterozygosity; P, proportion loci polymorphic; n_a, mean number of alleles per locus. Standard errors in parentheses.

any parametric test the significance of the regression statistics obtained. The slopes of the regression relationships, however, are useful descriptive measures of the inbreeding depression in each population. The effect of inbreeding on each population was assessed as the regression coefficient of each log-transformed demographic variable against the inbreeding coefficient of the litter, after removing the effects of litter parity, the inbreeding coefficients of dams and sires, and the age of the dam. This regression slope is the same as that used by Morton et al.[32] and Ralls et al.[40] to define the lethal equivalents per gamete with respect to juvenile survival. To examine the effects of inbreeding on the ability of dams to rear offspring, we used the partial regression coefficient of each demographic variable against the inbreeding coefficient of the dam. To compare inbreeding depression to initial levels of genetic variability in the stocks, we calculated Spearman rank correlations between the measures of genetic variation (percentage of polymorphic loci, number of alleles per locus, observed and expected heterozygosity) and the measures of inbreeding depression (regression slopes).

Results

Summary results of genetic variability observed in the wild-caught founder stock of lab populations are shown in Table 3. Five of the *P. polionotus* populations have been surveyed electrophoretically before by Selander et al.[46] and Garten.[17] Heterozygosity values for the I-SRI, P-LP, C-ONF, and C-LVY populations are in good agreement among the studies, and allele frequencies for enzymes tested in common are similar between Selander et al. and this study. For the I-SRI population, Garten reported 2.7% observed heterozygosity (H_o), Selander 2.0% expected heterozygosity (H_e), and this study 2.9% H_o and 5.3% H_e. For the P-LP population, Selander reported 9.3% H_e, this study found 8.7% H_e and 7.9% H_o, and Garten reported 5.9% H_o from Frostproof, 35 miles north of Lake Placid. For the C-ONF population, Garten reported 7.8% H_o, Selander 7.4%, 6.7%, 7.5%, and 8.1% H_e for sites in the Ocala area, and this study 6.0% H_o and 7.5% H_e. For the C-LVY population, Selander reported 9.5% H_e, this study 12.6% H_e. The Levy County site is near the contact zone between subspecies *subgriseus* and *rhoadsi*, and the higher levels of heterozygosity in that population may reflect genic interchange between the two forms. For the I-ANA population, the present values of 4.0% H_e and 4.2% H_o are much lower than previous reports of 7.8% H_e (Selander) and 9.1% H_o (Garten). Whether this is due to different subpopulations being sampled, sampling error, temporal fluctuation in allele frequencies, or a steady decline in genic variability is unknown. The I-ANA mice in the present study were collected from Conch Island, a manmade extension of Anastasia Island. Beach mice colonized Conch Island sometime in the past 50 years, and the amount of migration across the connecting isthmus is unknown. Moreover, the Anastasia Island population of beach mice has contracted considerably during the past few decades, and the remaining beach mice are found primarily in two small parks at opposite ends of the 22-km island.

Total sample sizes, mean values (± SE) of demographic variables on the outbred control mice, and greatest cumulative level of inbreeding achieved during the course of the experiment are given for each population in Table 4. Differences among populations in juvenile survival were due primarily to greater rates of infanticide by dams in some populations (especially I-ANA).

Regression coefficients for the effects of litter F, dam F, and sire F on each demographic variable (with effects of parity and age of the dam removed) are given in Table 5. The inbreeding of the litter had a negative impact on most demographic variables in most populations, though the magnitudes of these effects were often small. Even after achieving *F* values of .39 to .72, the regression coefficients measuring the effects of litter F were more than twice the standard errors in only 16 of 40 (nonindependent) comparisons. (Accurate parametric tests of statistical significance are not possible because only offspring mass can be used to approximate a normally distributed variable.) The effects of inbreeding of the sire and dam were inconsistent and typically within one standard error of zero. The slopes between parental F and the litter performance variables were greater than two standard errors in a few cases: a positive relationship between dam F and offspring mass in the C-NY population, a negative association between dam F and most demographic variables in C-TX, and a positive relationship between sire F and most demographic variables in P-CNS. Although we do not present the data in Table 5, first litters were consistently smaller, with higher mortality and slower growth rates, than were later litters. The age of the dam often, but not always, negatively affected the demographic measures. Sex ratios of mice at weaning were not significantly different from 1:1 in any population, and there were no significant differences between the weights of females and males at weaning. Inbreeding did not significantly affect sex ratio in any population.

Models including interaction effects (e.g., litter F × dam F) were also examined. Interaction effects did not contribute substantially to the variance in any de-

Table 4. Data on inbred and control outbred pairings of mice

Population	Outbred and inbred mice: No. pairs/ litters/weaned	Maximum F	Means for outbred controls[a]: Litter size	No. weaned	Viability[a]	Offspring 20-day mass	Litter mass[b]
I-ANA	385/249/353	.47	3.94 (0.18)	1.51 (0.33)	.40 (0.08)	8.48 (0.40)	11.77 (2.61)
I-SRI	166/0/143	.56	3.80 (0.42)	3.00 (0.70)	.70 (0.15)	7.66 (0.50)	23.39 (5.79)
P-LP	290/208/410	.48	3.89 (0.20)	2.38 (0.26)	.60 (0.06)	7.56 (0.13)	17.72 (1.99)
P-CNS	694/495/1,145	.63	4.05 (0.09)	2.65 (0.15)	.64 (0.03)	9.02 (0.13)	22.75 (1.41)
C-ONF	256/149/426	.39	3.34 (0.19)	2.41 (0.27)	.65 (0.06)	8.13 (0.24)	19.10 (2.28)
C-LVY	301/190/333	.43	3.30 (0.24)	2.11 (0.35)	.56 (0.09)	8.18 (0.23)	17.11 (2.97)
C-TX	1,159/812/2,347	.66	4.23 (0.10)	3.53 (0.14)	.83 (0.03)	12.45 (0.20)	43.10 (1.93)
C-NY	553/299/824	.72	3.79 (0.13)	3.16 (0.19)	.81 (0.04)	11.71 (0.22)	36.74 (2.30)

Standard errors in parentheses.

[a] N weaned ÷ litter size.

[b] Offspring mass × number weaned.

mographic variable. Nonlinear models (polynomial regressions) did not substantially improve the fit to the data for any variable. Very small first-order autocorrelations between residuals further confirmed that no nonlinear trends were overlooked. The regression relationships of each demographic variable against the inbreeding coefficient of the litter, across the ranges of F values produced in the experiment, are shown in Figure 1.

Table 6 gives the Spearman rank correlations among the various measures of inbreeding. Above the diagonal are correlations among the slopes of demographic variables against litter F (from the multivariate model incorporating litter F, dam F, sire F, parity, and age of dam). Below the diagonal are correlations among the slopes of demographic variables against dam F. On the diagonal are correlations for each demographic variable of the litter effect vs. the dam effect. Table 7 gives the Spearman rank correlations among the (negative of the) slopes in Table 5 and the measures of genetic variation in Table 3. Positive correlations indicate that the populations with the most allozymic variation were those displaying the strongest inbreeding depression (a steeply negative slope).

Table 5. Regression coefficients for demographic variables (natural log transforms of litter size, number of offspring weaned, viability to weaning at 20 days, mean offspring mass at weaning, and litter mass at weaning) versus inbreeding coefficients (F) from multivariate models incorporating effects of parity (first vs. later litters), dam F, sire F, and age of the dam

Population	Litter size	No. weaned	Viability	Offspring mass	Litter mass
I-ANA					
A	1.42(0.12)	0.60 (0.20)	0.24 (0.09)	2.13 (0.10)	1.19 (0.45)
B(F-lit)	−0.76 (0.21)	0.04 (0.36)	0.07 (0.16)	−0.10 (0.18)	0.15 (0.81)
B(F-dam)	−0.09 (0.25)	0.06 (0.42)	0.01 (0.19)	0.03 (0.18)	−0.20 (0.94)
B(F-sire)	−0.40 (0.29)	−0.93 (0.48)	−0.28 (0.22)	0.03 (0.24)	−1.56 (1.09)
R^2	.110	.055	.039	.012	.056
I-SRI					
A	1.69 (0.33)	0.79 (0.41)	0.22 (0.17)	2.11 (0.21)	1.41 (0.88)
B(F-lit)	−0.70 (0.74)	−1.56 (0.96)	−0.67 (0.40)	−0.71 (0.30)	−2.77 (2.10)
B(F-dam)	−0.13 (0.96)	1.79 (1.23)	0.82 (0.51)	−0.29 (0.39)	1.89 (2.85)
B(F-sire)	−0.35 (0.78)	−0.33 (1.00)	0.02 (0.42)	−0.44 (0.33)	1.27 (2.38)
R^2	.079	.239	.252	.713	.277
P-LP					
A	1.38 (0.14)	1.18 (0.21)	0.53 (0.10)	2.00 (0.06)	2.55 (0.46)
B(F-lit)	−1.19 (0.29)	−0.43 (0.45)	0.02 (0.21)	−0.08 (0.12)	−0.44 (0.99)
B(F-dam)	0.08 (0.39)	0.62 (0.61)	0.27 (0.28)	−0.09 (0.17)	1.01 (1.33)
B(F-sire)	−0.01 (0.43)	−0.63 (0.67)	−0.21 (0.31)	0.09 (0.19)	−1.14 (1.46)
R^2	.205	.108	.083	.018	.101
P-CNS					
A	1.47 (0.07)	1.15 (0.14)	0.49 (0.06)	2.16 (0.04)	2.54 (0.31)
B(F-lit)	−0.49 (0.13)	−1.01 (0.27)	−0.39 (0.11)	−0.23 (0.07)	−2.33 (0.61)
B(F-dam)	−0.26 (0.15)	−0.23 (0.29)	−0.06 (0.13)	−0.02 (0.08)	−0.43 (0.67)
B(F-sire)	0.14 (0.17)	0.91 (0.34)	0.41 (0.14)	0.21 (0.09)	2.22 (0.78)
R^2	.136	.041	.032	.053	.038
C-ONF					
A	1.50 (0.14)	1.50 (0.20)	0.63 (0.08)	2.12 (0.06)	3.25 (0.44)
B(F-lit)	−0.49 (0.34)	0.04 (0.46)	0.18 (0.20)	−0.10 (0.13)	0.69 (1.06)
B(F-dam)	0.32 (0.39)	0.44 (0.53)	0.12 (0.23)	0.00 (0.17)	0.50 (1.28)
B(F-sire)	0.00 (0.39)	0.29 (0.53)	0.22 (0.23)	0.08 (0.16)	0.92 (1.23)
R^2	.099	.121	.111	.068	.131
C-LVY					
A	1.45 (0.16)	0.92 (0.23)	0.36 (0.10)	2.03 (0.09)	1.70 (0.50)
B(F-lit)	−0.37 (0.30)	−0.35 (0.43)	−0.14 (0.19)	−0.36 (0.16)	−0.48 (0.97)
B(F-dam)	−0.09 (0.33)	−0.60 (0.47)	−0.25 (0.21)	0.18 (0.20)	−1.88 (1.07)
B(F-sire)	−0.26 (0.40)	0.51 (0.57)	0.18 (0.26)	−0.07 (0.22)	1.29 (1.29)
R^2	.064	.069	.055	.082	.066
C-TX					
A	1.61 (0.06)	1.73 (0.10)	0.70 (0.04)	2.56 (0.04)	4.11 (0.24)
B(F-lit)	−0.58 (0.11)	−0.95 (0.17)	−0.32 (0.07)	−0.25 (0.06)	−2.16 (0.43)
B(F-dam)	−0.38 (0.12)	−0.65 (0.19)	−0.28 (0.08)	−0.08 (0.07)	−1.47 (0.46)
B(F-sire)	0.09 (0.13)	0.13 (0.20)	0.08 (0.08)	0.10 (0.07)	0.37 (0.49)
R^2	.144	.139	.093	.057	.120
C-NY					
A	1.42 (0.10)	1.49 (0.15)	0.66 (0.07)	2.48 (0.05)	3.64 (0.37)
B(F-lit)	−0.31 (0.19)	−0.45 (0.28)	−0.24 (0.12)	−0.34 (0.09)	−1.16 (0.70)
B(F-dam)	−0.29 (0.18)	−0.38 (0.28)	−0.11 (0.12)	0.32 (0.08)	−0.53 (0.69)
B(F-sire)	0.21 (0.20)	0.11 (0.31)	0.01 (0.14)	−0.10 (0.10)	0.11 (0.77)
R^2	.063	.079	.076	.117	.074

In each case, regression coefficients listed (from top to bottom) are the constant term (A, for multiparous females and dam age = 0); slopes, B, for litter F, dam F, and sire F; and R^2, the proportion of variation accounted for by the model. Standard errors in parentheses.

Discussion

When a population goes through a bottleneck, declining to numbers that make inbreeding likely or even inevitable, formerly rare deleterious alleles undergo one of two fates: either they are excluded from the population by chance or they become less rare (persistence in a very small population implies presence within that sample at a greater frequency than does persistence in a large population) and are exposed more often as homozygotes during the generations that the bottleneck persists than they were when the population was large. The frequency of recessive, deleterious alleles expected under selection-mutation equilibrium with no complicating effects of drift is given by q = sqrt(m/s), with mutation rate m and selection coefficient s. Yet such very low frequencies (on the order of 10^{-3} for typical mutation rates) cannot be maintained and may not be possible in very small populations subjected to random drift. As a result, the mean frequency of recessive, deleterious alleles maintained in small populations is an order of magnitude or more smaller than in large populations.[33,44,56] The bottleneck need not be severe to cause a substantial reduction in the equilibrium frequency of recessive lethals. Even populations of an effective size of 1,000 maintain only about one-tenth the load of recessive lethals of an effectively infinite population.[10] Blair[4] estimated the total population size of *P. polionotus leucocephalus* on Santa Rosa Island (I-SRI population) in 1941–42 to be about 12,000. As the genetically effective population size would be a fraction of that number, this island-endemic subspecies would likely maintain a reduced load of lethals even in the absence of further bottlenecks caused by hurricanes, cat predation, and habitat loss. Because of the random loss of rare variants in small to medium populations, the lethal load of such a population at equilibrium would decline more rapidly with population size than does heterozygosity (which is determined primarily by the midfrequency alleles).

During severe bottlenecks, when inbreeding may be common but the population is far from genetic equilibrium, each rare deleterious recessive allele will be expressed in homozygotes with a frequency of about Fq, in which F is the mean in-

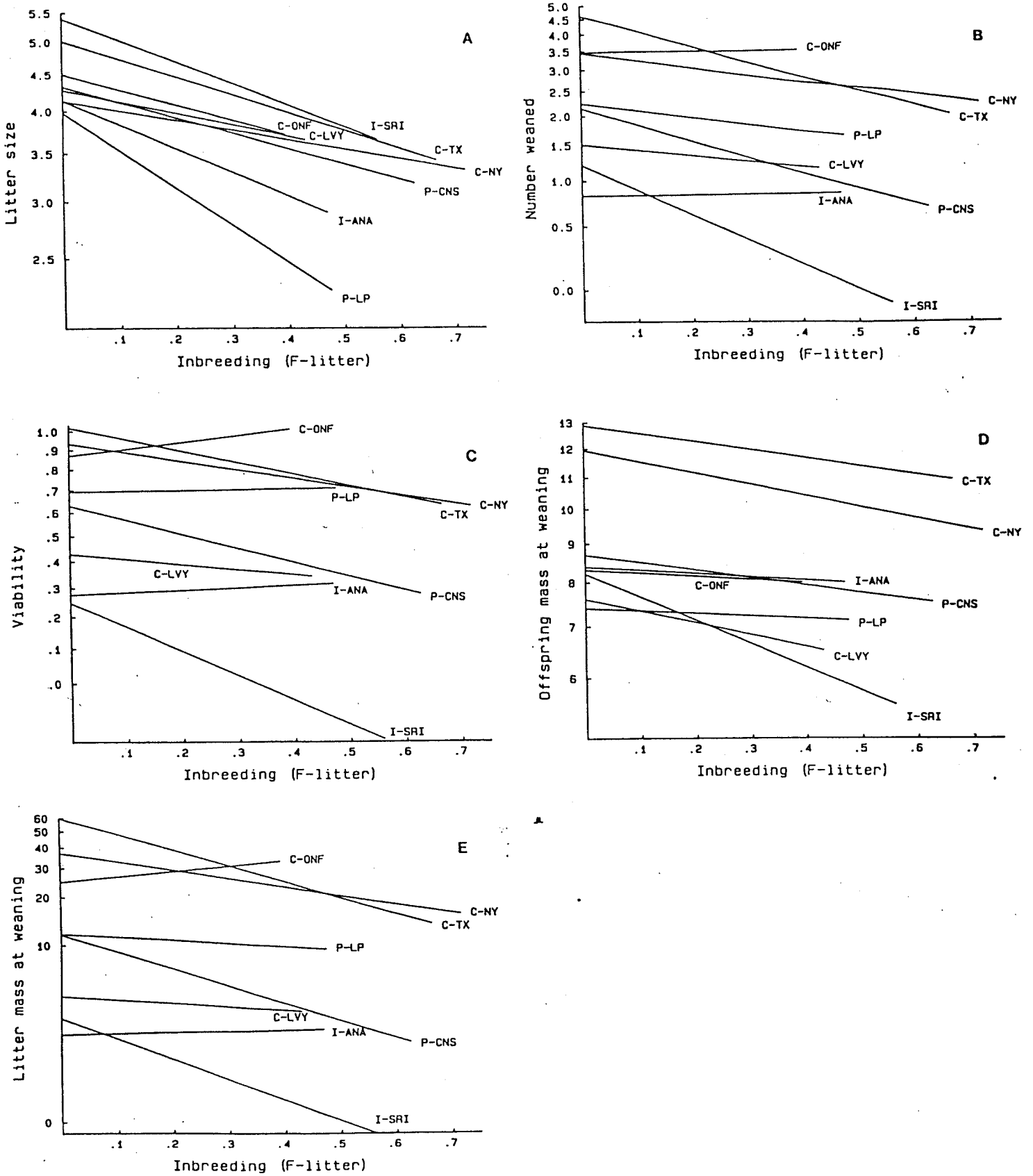

Figure 1. Least squares regression lines for the relationships between various demographic variables vs. the inbreeding coefficient of the litter, for eight populations of *Peromyscus* mice. Demographic variables examined are (A) initial size of litters (log-transformed), (B) number of offspring weaned [ln(no. weaned + 1)], (C) viability of litters [ln(no. weaned/no. born + 1)], (D) mean mass offspring at weaning [ln(grams)], and (E) litter mass at weaning [ln(grams + 1)]. The regression model included also as independent factors the inbreeding coefficients of the sire and dam, the age of the dam, the dam parity (first vs. later litters). The y-axis intercepts of the lines in the figure were obtained from the multivariate model for multiparous dams of age 0, with inbreeding coefficients of sire and dam set to 0.

Table 6. Rank correlations among measures of inbreeding depression

	Litter size	No. weaned	Viability	Offspring mass	Litter mass
Litter size	−.19	.10	−.05	−.43	−.05
No. weaned	.55	−.02	.98*	.55	.98*
Viability	.55	1.00*	.10	.69	1.00*
Offspring mass	−.02	−.55	−.55	−.10	.69
Litter mass	.43	.98*	.98*	−.64	.05

Above the diagonal, inbreeding depression is based on regression slopes against litter F (second row of each set in Table 5). Below the diagonal, inbreeding depression is based on slopes against dam F (third row of each set in Table 5). On the diagonal are rank correlations between these two measures of inbreeding depression for each demographic variable.

*Significant at $P < .001$.

breeding coefficient and q is the frequency of the allele. Selection will then eliminate some of those homozygotes, resulting in a reduction of the frequency of a rare deleterious allele of approximately Fqs, in which s is the selection intensity against the homozygote. The genetic load of a bottlenecked population, as measured by lethal equivalents, would therefore be expected to be reduced by fraction F, directly proportional to the expected reduction in heterozygosity. Thus, if differences in heterozygosity among some populations are due solely to genetic drift during recent bottlenecks, and if the populations contained the same lethal equivalents prior to the bottlenecks, then the lethal equivalents in the populations following bottlenecks should be approximately proportional to the remaining heterozygosities. As the populations subsequently expand, new mutations would restore heterozygosity and genetic load. The rate of restoration of genetic variation depends on the selection-mutation balance; neutral variation would return at a rate equal to the mutation rate, and selectively disadvantageous variants would be restored more slowly (as some new mutants were removed by selection). Thus, the restoration of the genetic load of a population following a bottleneck should occur more slowly than the restoration of nonlethal recessive variants, and also more slowly than allozymic variation. Considering both the selection-mutation-drift equilibrium and the rate of loss during sudden bottlenecks, therefore, electrophoretic measures of heterozygosity of neutral or near-neutral allozymes would be expected to be monotonically related to the genetic load of recessive deleterious alleles, though would likely be less sensitive to population size and bottlenecks than would the genetic load.

Table 7. Rank correlations among measures of genetic variation and inbreeding depression

	Litter size	Number weaned	Viability	Offspring mass	Litter mass
H_e	−.69	.12	.21	.36	.21
	.33	.76*	.76*	−.45	.81*
	−.60	−.60	−.36	−.07	−.36
H_o	−.64	.00	.05	.10	.05
	.21	.60	.60	−.60	.67
	−.67	−.48	−.21	−.12	−.19
P	−.37	−.37	−.31	−.12	−.31
	−.11	.64	.64	−.24	.64
	−.30	−.46	−.35	−.34	−.11
n_e	−.52	−.46	−.37	−.07	−.37
	−.19	.62	.62	−.39	.65
	−.30	−.52	−.40	−.22	−.17

In the first, second, and third rows of each set inbreeding depression is based on regression slopes against litter F, against dam F, and against sire F, respectively (corresponding to the three rows of slopes in Table 5). In each case dam parity and dam age were included as independent factors in the multivariate model.

*Significant at $P < .05$.

If inbreeding depression results from overdominance at loci affecting fitness traits, the consequences of bottlenecks are more difficult to predict. Selection against both homozygotes, while resulting in inbreeding depression, could not remove the genetic load directly. During prolonged inbreeding, selection at modifier loci could lessen the heterozygote advantage, however. Island and peripheral populations therefore may show less inbreeding depression due to heterotic loci than would central populations adapted to high heterozygosity. Alternatively, if fitness (perhaps dependent upon developmental homeostasis) declines more rapidly than heterozygosity during inbreeding, then island populations already depleted of genetic variation could possibly suffer even more from a decline in heterozygosity during inbreeding than would central populations with greater initial heterozygosity.

Much of the genetic load of a population could consist of mildly deleterious, partially dominant alleles. In the absence of inbreeding, selection against such alleles acts primarily on heterozygotes (because homozygotes are much rarer), and the equilibrium frequency of partially dominant deleterious alleles is almost independent of population size when the effective population size is on the order of 500 or more.[33] Selection against homozygotes during more severe bottlenecks does remove partially dominant deleterious alleles, and this component of the genetic load would respond as do fully recessive alleles to severe bottlenecks and inbreeding.

The eight populations of *Peromyscus* mice studied here showed levels of electrophoretically detectable genetic variation that were in accord with the presumed past histories of bottlenecks. The isolated island populations inhabiting frontal dunes highly susceptible to damage by hurricanes (I-SRI and I-ANA) had about one-third to one-half the heterozygosity that was observed in more central, nondune, and much larger populations of that species (C-LVY and C-ONF) and of congeners (C-NY and C-TX). Moreover, the island populations especially lacked uncommon variants (as reflected in the lower number of alleles per locus and the fewer polymorphic loci), which would be eliminated during bottlenecks much more rapidly than is heterozygosity.[1,16] The lower genetic variation in the I-ANA population relative to that observed 15 years earlier also might be a consequence of the rapid decline in range and numbers over the past few decades. The peninsular and peripheral populations that have suffered moderate range contractions (P-CNS and P-LP) displayed intermediate levels of heterozygosity. Although not in perfect accord with presumed population sizes and vulnerability to bottlenecks (e.g., the C-ONF population occupies habitat as extensive as and perhaps contiguous with that of the C-LVY population but may have less genetic variability), the levels of allozyme variation reflect well the presumed impacts of genetic drift. Unfortunately, without detailed knowledge of the breeding systems of the populations sampled, we cannot be certain that inbreeding has been more common in the island populations than in the mainland populations. We have no evidence of localized inbreeding or population substructuring within the areas trapped: in none of the populations sampled were genotype frequencies significantly out of Hardy-Weinberg-Castle equilibrium at the loci examined, though our small sample sizes would preclude detection of low levels of inbreeding. The deficiency of heterozygotes observed in the C-TX population, although not statistically

significant, may have resulted from our sampling over a wider area than with other populations; similarly, the deficiency of heterozygotes in the I-SRI population could reflect partial isolation of the mice inhabiting the two sections of protected national seashore on Santa Rosa Island.

Selection, rather than drift, could have caused the observed differences in genic diversity if allozymes are not selectively neutral. Loudenslager[25] suggested that geographically wide-ranging *Peromyscus* taxa maintain greater heterozygosities because of adaptation to more variable environments and that insular species are often restricted to narrow habitats, but Smith[50] considered species differences in heterozygosity more likely to be due to the frequency of and the rate of recovery from population bottlenecks. Differences in habitat diversity would be unlikely to account for the lesser heterozygosity of the I-ANA and I-SRI populations relative to the P-CNS population in a similar frontal dune habitat.

The two species of mice studied probably predominantly outcross in the wild. Patterns of dispersal are sufficient to minimize inbreeding,[5,55] and behavioral inbreeding avoidance has been reported in *Peromyscus*.[12,22] *P. polionotus* seems to form long-term monogamous pair bonds,[14] and at least in the C-ONF population there is no evidence that the monogamous pairs deviate from random mating.[15]

The responses to inbreeding in the lab were quite varied. In some populations litters were smaller at birth, others had higher juvenile mortality, others slower growth, and one (C-ONF) showed no significant response to inbreeding in any of these fitness traits. Except for those pairs of demographic variables that measure overlapping aspects of fitness (litter viability and litter size at weaning, offspring mass and litter mass, litter size at weaning and litter mass at weaning), the demographic variables responded independently to inbreeding (Table 6). Lab stocks founded with samples from different subspecies of a species and even with samples from populations of a single subspecies collected about 50 miles apart (C-ONF and C-LVY) expressed inbreeding depression in different traits and to different degrees. The genetic loads, as measured by lethal equivalents per gamete (negative slope of regression lines for viability versus litter F) were smaller than reported for many other homeotherms, ranging from −.18 to .67. With one exception, the values reported here fall within the lower quartile of lethal equivalents reported by Ralls et al.[40]

Other researchers have examined inbreeding in *Peromyscus*, reporting responses as disparate as those in the present study. *Hill*,[22] studying *P. maniculatus*, and Dewsbury,[12] studying *P. maniculatus* and *P. eremicus*, found lower rates of reproduction by sibling pairs than by nonsibs. The difference lessened if the sibs were isolated for 50 days (Hill) or 24 days (Dewsbury) before pairing, suggesting a behavioral avoidance of inbreeding that diminished with loss of familiarity. Hill[22] reported that the inbred litters had fewer young, lower individual weights, and higher mortality than did noninbred litters, whereas Dewsbury[12] observed no effect of inbreeding on initial litter size or number of pups weaned. Skryja[48] observed no reduction in fertility of father-daughter pairs of *P. eremicus* housed together continuously. Haigh[19] observed smaller weights and lower survival to weaning among offspring of father-daughter pairs of *P. maniculatus*, but no effect on number of litters produced or litter size at birth.

The deleterious effects of inbreeding can occur at two levels: inbred offspring may suffer reduced fitness because of their increased homozygosity, or the manifestation of inbreeding depression may be delayed one generation if inbred animals are poor parents, failing to rear litters as large and healthy as those of outbred parents. For no demographic variable did we find a between-population association between the depression of fitness resulting from the inbreeding of the dam and the depression resulting from the inbreeding of the litter itself (Table 6): these two levels of inbreeding would seem to be determined independently.

Of the 20 nonindependent correlations examined between a measure of genetic variability in the founder stock and the effect of inbreeding on the litter, only eight were positive, none significantly so (Table 7). Thus, there was not an overall trend for litters in the genetically depauperate insular populations to suffer less from inbreeding depression, as would be expected if past bottlenecks reduce genetic load while reducing allozyme variation. It is possible that none of the populations studied have experienced bottlenecks sufficiently narrow to remove a genetic load of recessive alleles, especially if much of the load was due to alleles with small deleterious effects on heterozygotes. Observed differences in heterozygosity and allelic diversity (Table 3), as well as differences in the genetic load expressed in inbred litters, could have had causes unrelated to population size.

The impact of inbreeding on maternal performance, measured by the effects of dam F on size, growth, and survival of the litter, was substantial in only one of the populations (C-TX). Viability (thus also the number weaned and litter weights) was markedly depressed in the litters of inbred dams from the genetically variable C-TX stock, with a suggestion of the same trend in the C-LVY and C-NY stocks. As a result of the inbreeding depression affecting dams in the most variable populations, 14 of 20 nonindependent correlations between measures of genetic variability and the effect of dam F on litter performance were positive, three significantly so (Table 7). Selection in the less variable and more isolated populations may have reduced the load of recessive genes that would, when homozygous, diminish the ability of a female to rear offspring.

The considerable variability and almost randomness observed in the responses to inbreeding, with respect to both which populations showed strong inbreeding depression and which fitness traits were affected, suggests that the deleterious effects of inbreeding result from selection on relatively few genes. The varied responses can best be interpreted as being due primarily to historical accident, not predictable differences in the genetic composition of the populations. We cannot determine whether the differences observed among our lab populations are due to differences in susceptibility to inbreeding depression among the wild populations from which they were derived or whether the differences resulted from the stochastic nature of sampling wild populations to initiate lab stocks. The numbers of founders used in this experiment (five to 15) are probably not unlike the numbers that colonize vacant habitats to establish new natural populations, and may be more than the founding stocks of many island populations. Replicate experiments on multiple subsamples of each wild population would be needed to assess the stage at which randomness in inbreeding response originates.

Although responses by the populations to inbreeding in the laboratory were diverse, within each population trends were more consistent. Demographic variables responded linearly to inbreeding (polynomial regression models did not improve the fit to the data), and preliminary results from early generations of lab breeding[7]